

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization International Bureau



08 JUL 2005



(43) International Publication Date
5 August 2004 (05.08.2004)

PCT

(10) International Publication Number
WO 2004/064744 A2

(51) International Patent Classification⁷:

A61K

(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(21) International Application Number:

PCT/US2004/001334

(22) International Filing Date: 16 January 2004 (16.01.2004)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

60/440,792

16 January 2003 (16.01.2003) US

(71) Applicant (for all designated States except US): GENENCOR INTERNATIONAL INC. [US/US]; 925 Page Mill Road, Palo Alto, CA 94117 (US).

(72) Inventors; and

(75) Inventors/Applicants (for US only): LEEFLANG, Chris [NL/NL]; Granaathorst 345, NL-2592SZ The Hague (NL). VAN DER KLEIJ, Wilhelmus, A., H. [NL/NL]; Lann van Waterungseveld, NL-2548CZ The Hague (NL).

(74) Agent: ITO, Richard, T.; Genencor International, Inc., 925 Page Mill Road, Palo Alto, CA 94304 (US).

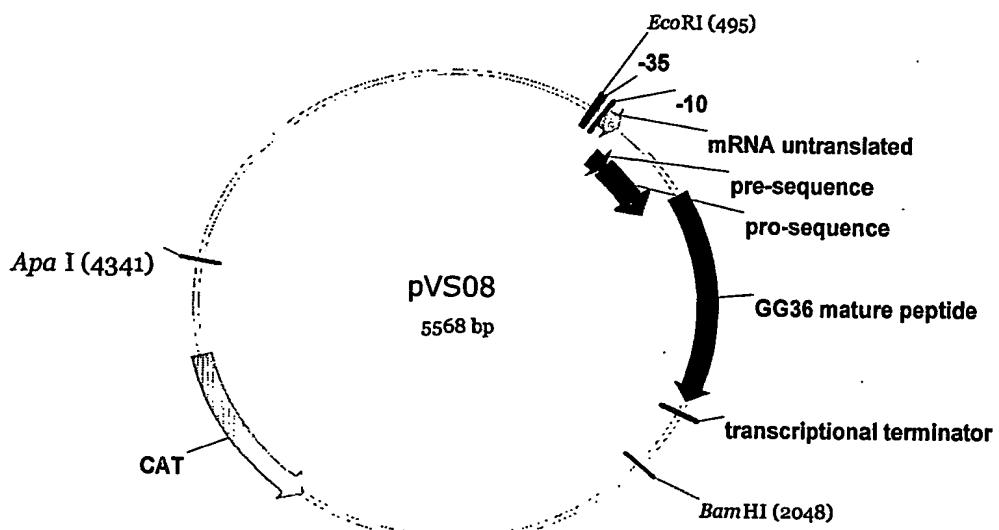
(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

— without international search report and to be republished upon receipt of that report

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: METHODS FOR SITE-DIRECTED MUTAGENESIS AND TARGETED RANDOMIZATION



WO 2004/064744 A2

(57) Abstract: The present invention provides methods and compositions for the construction and direct transformation of site-saturation libraries into *Bacillus*. This method avoids the need for the use of intermediate hosts, such as *E. coli* for the development of *Bacillus* strains suitable for the production of proteins.